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EFFICACY OF *LEPIDIUM SATIVUM* SEEDS AGAINST CARBON TETRA CHLORIDE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT : The aim is to assess the hepatoprotective and antioxidant effect of *Lepidium sativum* extract against different liver damage caused by carbon tetrachloride. We used white rats in this study. The included rats were divided into two groups, the case group and the control group. We injected the rats of the case group with 0.1 ml of CCl_4 per 100 gram body weight mixed with the same amount of olive oil intraperitoneally two times weekly and treated them with (LSS) in a dose of 200 mg/kg daily for twelve weeks. The rats in the control group were injected with 0.1 ml of CCl_4 per 100 gram body weight mixed with the same amount of olive oil intraperitoneally two times weekly for twelve weeks. CCl_4 administration resulted in marked changes in the serum levels of liver enzymes, including (GOT, GPT and ALP) and oxidant substances, including (MDA and CAT), which indicates that it caused liver injury. In addition, our results showed that LSS extract administration resulted in a marked decrease inthe GOT, GPT and ALP levels and increase in the antioxidants, including glutathione peroxidase, catalase and reduced glutathione levels in the treated group compared to control group. In addition, the results of histopathological examination of the liver showed degenerated liver cells, thickened central vein walls and the presence of inflammatory cells in the portal tracts and central veins in the ratsin the control group. However, these changes improved markedly in those of the case group which received LSS, where they showed reduction in the degeneration and necrosis of the liver cells. This means that treatment with LSS in a dose of 200 mg/kg and followed by CCl_4 injection protects against its nuclear degeneration effect.

We conclude that LSS extract has a protective role against CCl_4 toxic effect. So, it can be used as a prophylactic agent against variantliver injuries induced by CCl_4.

Key words : *Lepidium sativum*, antioxidants, carbon tetrachloride.

INTRODUCTION

The environmental pollutants, toxic chemicals and drugs cause cellular damage by activation of reactive oxygen species (Sies, 2019). Carbon tetrachloride (CCl_4) is one of toxic substance that used in inducing hepatotoxicity in lab animal also, causes damage in many organs such as, heart, testis, brain and kidney (Fahmy *et al*, 2018).

According to several studies, CCl_4 showed that have great negative effect on many organs such as brain, kidneys, liver, testis, heart, lung and blood. Also, CCl_4 causes an acute and chronic lesion in several organs (Negi *et al*, 2018).

The medicinal plants were used for treatment of differental infections and diseases. Plant treatment (Phytotherapy) is considered the oldest methods used to cure the diseases. Pure (extracts) that isolated from plants are used to treat different diseases such as cardiovascular diseases, jaundices, diabetes, scarlet fever, abdominal and pelvic diseases and to treat most heavy metal poisoning. (Shahid *et al*, 2017).

Lepidium sativum Linn (El-Rashad) is fast-growing edible herb, it hastangy and aroma flavor (Hussein *et al*, 2017). Several active constituents of the plant are used for treating liver disease, gastrointestinal diseases, jaundice, spleen diseases, menstrual problems, fracture and arthritis. For example, *L. sativum* is used to treat headache, the uterine tumor, nasal polyps and breast cancer (Fahmy *et al*, 2018). *L. sativum* contains some essential fatty oils, protein, vitamins, isothiocyanates glycoside, carbohydrate and flavonoids. Many previous studies showed that *L. sativum* has different actions such as: antioxidant effect, antihypertensive effect, anti-inflammatory effect, anti-asthmatic effect, hypoglycemic effect and diuretic effect (Emhofer *et al*, 2019). Using LSS as protective substances and antioxidant agents to stop some physiological and biochemical changes in rat that injected by carbon tetrachloride are the purpose of our study.

MATERIALS AND METHODS

Collection of plant material

The local market is provided with our study with LSS in Ramadi, Iraq and was kept at airtight closer containers.

Extract preparation

Soxhlet apparatus is used for plant extraction by taking 50 seed and grind it to convert it into powder. Then the powder isliquefied into 500 ml alcohol (ethanol) for 6–8 hours. The extract is filteredand the solution for (Bauchi Company, Switzerland) is heated at (60^0C) and pressure (175 bar). The extract stored at optimum storage temperature (4^0C) (Al-Asmari *et al*, 2015).

The chemical compound

Carbon tetrachloride (CCL_4) was provided by one of the common pharmaceutical companies(Sigma Company) (USA).

Animals

30 Wistar ratshave a weight range (170–180 gram), it was given by the Faculty of Veterinary Medicine, University of Baghdad, central animal house. It keeps in cages at (22-28^0C) with a half of day (12) hours dark and the rest of the day (12) hour light and were supplied by standard water andfood. The experimental animals (Rats) are divided in (6) groups. The first group isthe control group and provided water and pellets for (12) weeks. The second group was provided with CCl_4 (0.1) ml/100gram B.W with (50% in olive oil) intraperitoneal twice a week for (12) weeks. The third group was provided with (1) ml/kg olive oil intraperitoneal twice a week for (12) weeks. The fourth group was provided with LSS extract (200) mg/kg B.W. taken orally for (12) weeks. The fifth group was provided with CCl_4 (0.1ml/ 100g B.W.) (50% in olive oil) intraperitoneal twice a week for 4 weeks and then provided LSS extract (200 mg/kg B.w.) orally for B.w. orally eight weeks. The sixth group was provided LSS extract (200) mg/kg B.w.Orally, after this,was provided with CCl_4 (0.1) ml/100g B.w. (50% in olive oil) Intraperitoneal 2 times a week for 12 weeks. The animals (rats) were sacrificed after anesthesia. 2 Ml blood sample was collected directly from the heart from all animals and thenleft to dry for produce serum then the centrifugation process was done, using separation 3000 RPM for 15 min. then, the serum was stored at high freezing degree (-20°C).

Serum biochemistry

The serum of glutamate pyruvate transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and alkaline phosphatase (ALP) were measured using Roche kits and Reflation plus Analyzer (Diagnostics GmbH of Roche, Mannheim, Germany). While the levels of urea and creatinine were measured using standard test kits (Laboratories of Randox, Crumlin, the County Antrim, Northern Ireland, UK).

Oxidative stress analysis

Lipid peroxidation was assessed by knowing MDA, GSH and CAT amounts. MDA level is measured depending on the manufacturer's instructions (Sigma-Aldrich company, Germany). GSH and CAT level were measured by using kits (Biodiagnostic-Sigma-Aldrich Company, Germany).

Histopathological examinations

First, making fixation of liver tissues by using formalin, as the sample embedded in paraffin for cutting (5) m in thickness. All samples are stained with hematoxylin and eosin (Sigma Company, Germany) using the microscope for reading the results.

Statistical analysis

Using ANOVA SYSTEM to analyse the results by Graph Prism Software Version. 7.00.

RESULTS

Liver enzymes and LSS

Carbon tetrachloride (CCL_4) caused increasing in the serum of liver enzymes (GPT, GOT & ALP), when we comparethe first Group (control) to group II (CCL_4). After using LSS in Groups (IV, V & VI), enzymes level was decreased at ($P \leq 0.05$) comparing to Group II.

Oxidative state results

As compared to the control group (Group I), the animals treated with CCL_4 (Group II) showed increasing in serum levelof MDA at ($p < 0.05$). While showing a decrease in GSH and CAT (%) levels, treatment with LSS extract showed decrease in MDA level and increased in GSH & CAT level. The activities of GSH & CAT have been enhanced after treatment with the LSS extract in group IV, V & VI.

Histopathological study

The control group (Group 1)and (group III) showed that the liver tissue is normal such as Figs. 7, 9). Sections of liver showed that liver cells are arranged around the central veins, having normal cytoplasm and prominent nucleus. But in the CCL_4 treated group (group II), its sections of liver showed degeneration and necrosis of the liver cells caused by infiltration with inflammatory cells as shown in Fig. 8. In the group treated by LSS (group IV), its sections of the liver showed a decrease in necrosis and degenerated hepatocytes (Fig. 10). In a group treated with LSS-CCL_4 (Group VI) showed

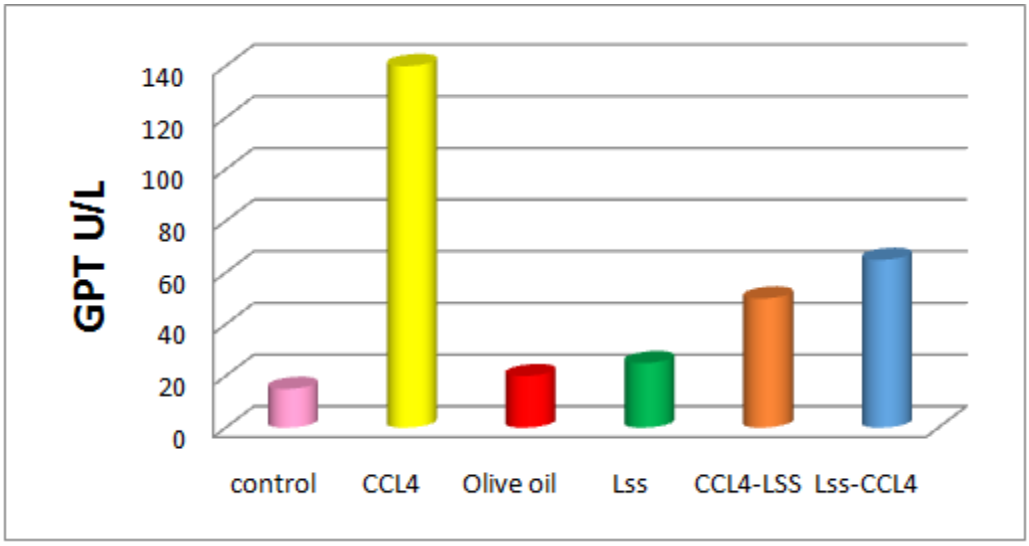

Fig. 1 : Treatment with ethanolic extract of LSS and its effect on (GPT) enzyme level in experimental rats treated with CCl_4, each group consists of five animals.

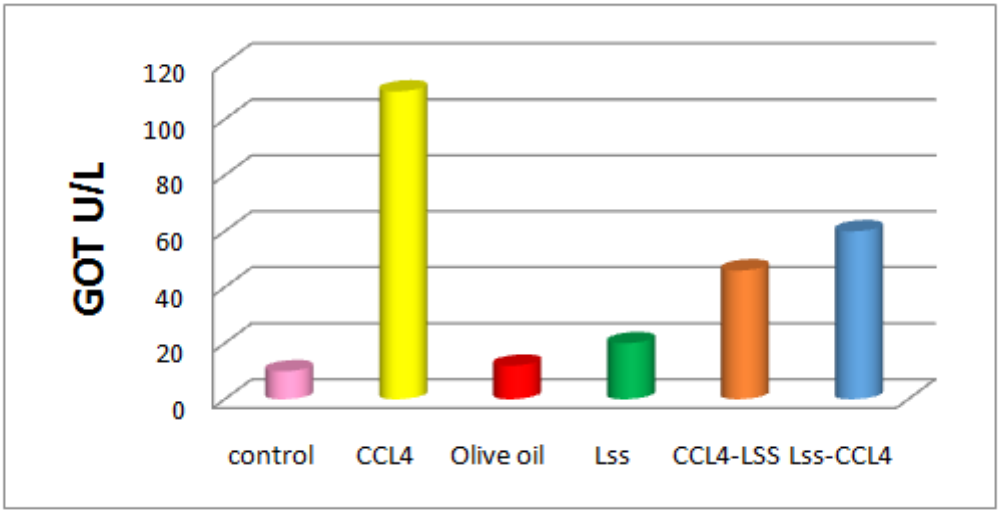

Fig. 2 : Treatment with ethanolic extract of LSS and its effect on (GOT) enzyme level in experimental rats treated with CCl_4, each group consists of five animals.

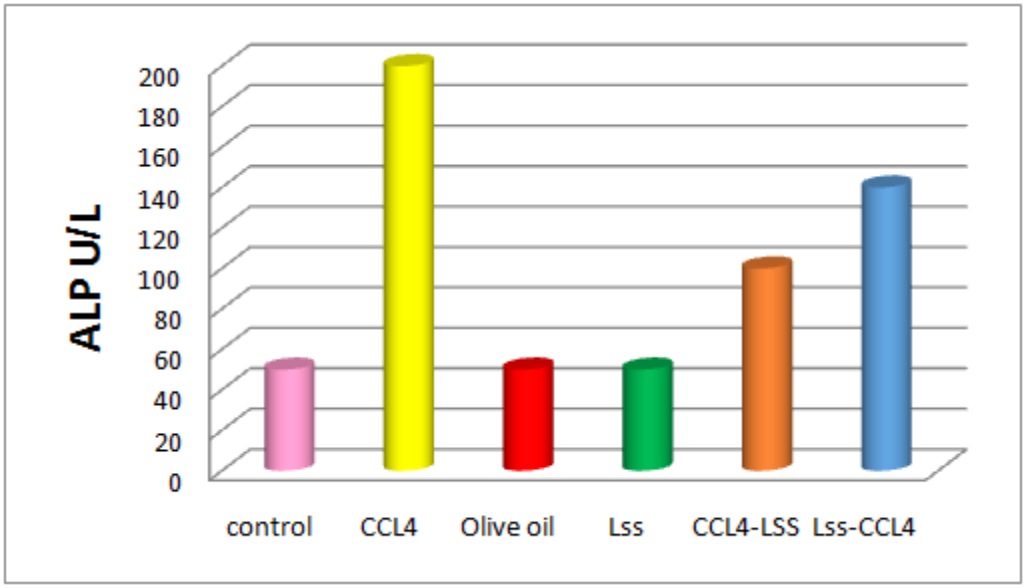

Fig. 3 : Treatment with ethanolic extract of LSS and its effect on (ALP) enzyme level in experimental rats treated with CCl_4, each group has five animals.

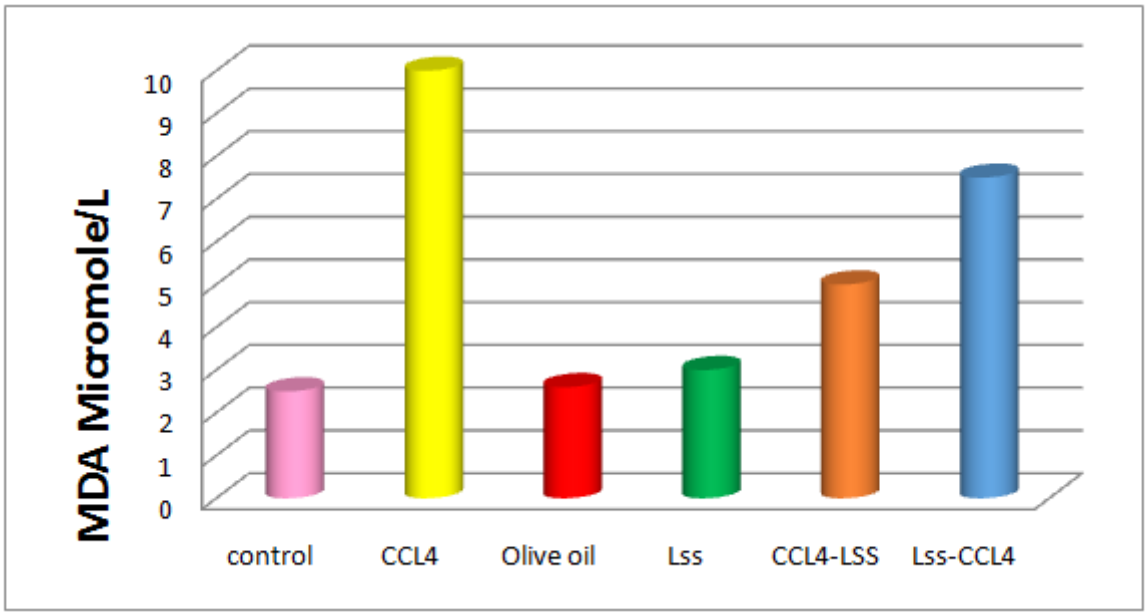

Fig. 4 : Treatment with ethanolic extract of LSS and its effect on (MDA) enzyme level in experimental rats treated with CCl_4, each group consists offive animals.

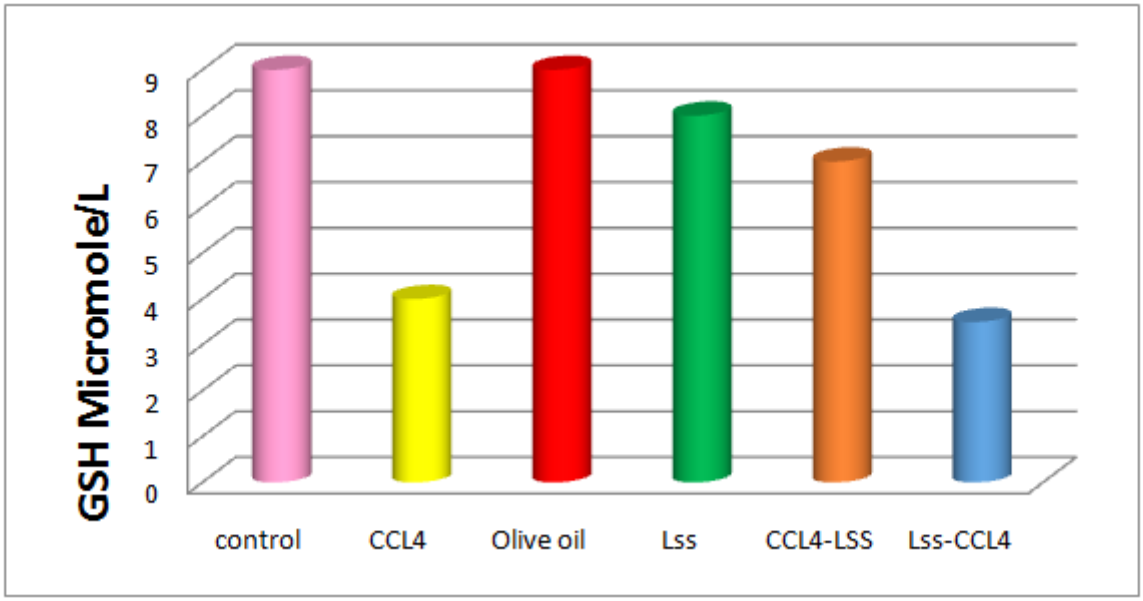

Fig. 5 : Treatment with ethanolic extract of LSS and its effect on (GSH) enzyme level in experimental rats treated with CCl_4, each group consists of five animals.

minimum degeneration inside the nucleus (Fig. 12). In a group treated by CCL_4- LSS (Group V) showed moderate nuclear degeneration (Fig. 11), this study showed that (LSS extract) has a significant role in decreasing CCl_4 toxicity.

DISCUSSION

Hepatic diseases are a public health problem and increasing nowadays due to increasing obesity in all age groups (Breda *et al*, 2019). The common treatments of liver diseases with drugs have many adverse effects (Doulberis *et al*, 2019), so, we can use medicinal plants (Pytotherapy) that contain natural substances with minimum side effect or without side effects (Doulberis *et al*, 2019).

The results of this study showed that *L. sativum* that extracted by ethanoic has hepatoprotective effect. Carbon tetra chloride (CCl_4) is considered a toxic substance in the cell; it converted to two compounds, Cl_3COO and trichloromethane (CCl_3) by cytochrome P-450 (C- p450) inthe endoplasmic reticulum (Li *et al*, 2019b). After conversion into its products, it makes several changes, Carbon trichloride (trichloro methane) (CCl_3) causes marked degeneration and necrosis of fatty layers in the liver (Ferreira *et al*, 2018). Carbon tetrachloride

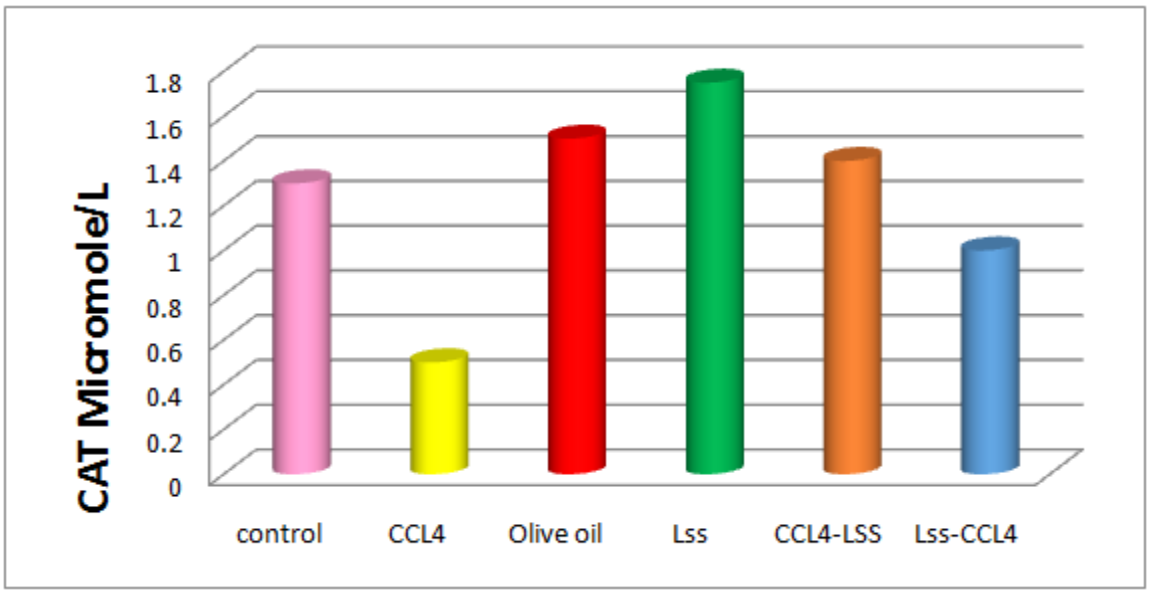

Fig. 6 : Treatment with ethanolic extract of LSS and its effect on (CAT) enzyme level in experimental rats treated with CCl_4, each group consists of five animals.

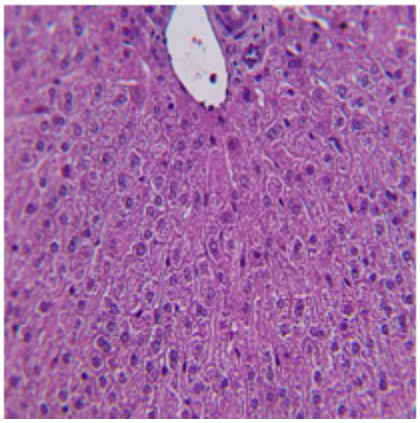

Fig. 7 : The liver of normal control rat (group 1) showing no histopathological changes.

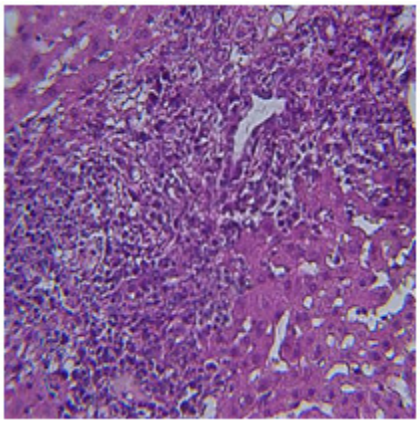

Fig. 8 : The Liver of CCl_4 group (group II) showing, inflammatory cells infiltration (MNC), thickness wall of Central vein (TW), increase in fibroblast (F).

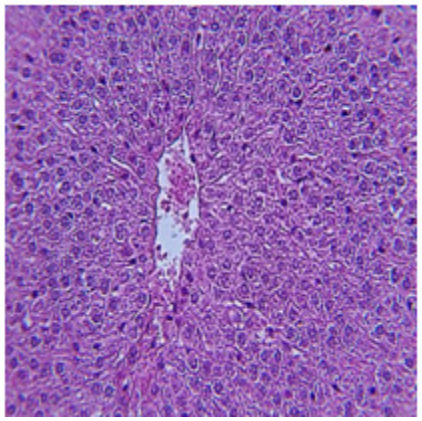

Fig. 9 : The liver of second normal control rat (group III) showing no histopathological changes.

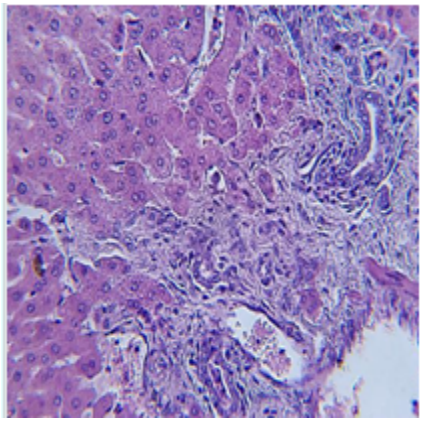

Fig. 10 : The liver of LSS group (group IV) showing no histopathological changes Central vein (CV), Hepatocyte (HC).

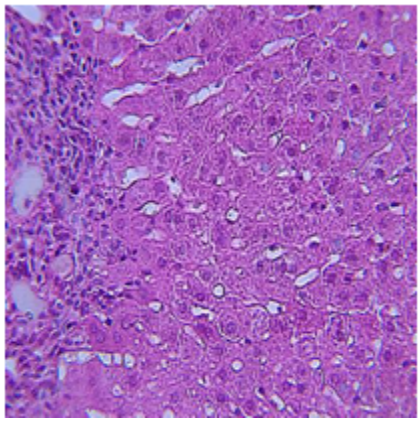

Fig. 11 : The liver of CCl_4 & LSS group (group V) showing inflammatory cells infiltration (MNC).

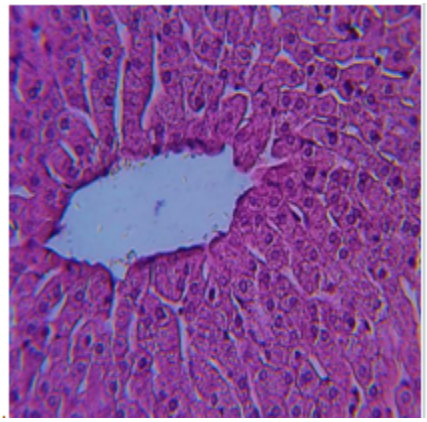

Fig. 12 : The liver of CCl_4 & LSS group (group VI) showing inflammatory cells infiltration (MNC).

(CCl_4) cause elevation of liver enzymes such as GPT, GOT & ALP. So, GPT & GOT level increase in the blood (which leaked from the damaged liver) (Salma *et al*, 2018).

Increasing the level of GOT may indicate to muscle injury, disorder of the liver and cardiac infarction. Increasing the level of ALP indicate internal or external biliary obstruction was caused by infiltration. In this study, the results showed that treatment with LSS have a hepatoprotective effect, decrease liver damage, improve histological structure and this result agrees with Chhipa and Sisodia (2019) many reports showed that LSS has an anti-oxidative effect due to presence of phenolic compounds in LSS composition, some studies approved that, the phenolic compounds have an anti oxidant activity towards the liver damage (Al-Asmari *et al*, 2015).

CCl_4 is one of the environmental toxicant to induce animal models of acute hepatic damages and nephrotoxicity experimentally. The administration of CCL_4 causes elevation in both levels of urea and creatinine in the experimental rats when compared with the control rats due to decree filtration rate. Nevertheless, after administration of LSS extract, the serum level of urea and creatinine decreased due to increase glomerular filtration rate. CCL_3 produced by conversion of CCl_4, causes hepatotoxicity (Slama *et al*, 2018), the lipid peroxidation caused membrane damage due to variant changes in the structureof cell membrane (Abdollahi *et al*, 2019). MDA is an enzyme produced through lipid peroxidation, used as an indicator of oxidative stress in hepatotoxicity by CCl_4 (Lee *et al*, 2019).

GSH and CAT are the most common endogenous antioxidants used for deactivation of free radicals (Jamor *et al*, 2019). CCL_4 causes depletion of GSH and CAT (Li *et al*, 2019a). Due to losing antioxidant enzymes, Free radicals will accumulate (Ikechukwu *et al*, 2019). Malondi-aldehyde (MDA) level increased after injection CCl_4, causing tissue damage due to losing of antioxidant defense. Finally, LSS causes reduction of MDA and increase in GSH & CAT levels. So it has an effective role in protecting against CCl_4 (Sangekar *et al*, 2018) and the histological observation of the groups treated with LSS showed a protective agents of the liver, the protective effect is including a decrease or (absent) ininfiltration and necrosis.

CONCLUSION

LSS extract has a protective role against CCl_4 toxic effect. So it can be used as a prophylactic agent against variant liver injuries induced by CCl_4.

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